AMENDMENT

In the Specification:

Please add the following description of Figures 2-9 to the Brief Description of the Figures, beginning on page 6, line 15:

--Figure 2 shows the construction of pSK – MUT, in which four PCR fragments were sequenced and pieced together to form the complete mutase gene in pSK-bluescript.

Figure 3 shows acyl-CoA analysis in BL21 (DE3) panD strains in vivo.

Figure 4 shows the results of CoA analysis of *E. coli* overexpressing methylmalonyl-CoA mutase. The levels of ^{3}H detected in fractions collected from HPLC of cell-free extracts from ^{3}H ß-alanine-fed *E. coli* harboring either the pET control vector grown without hydroxocobalamin (black trace), pET grown with hydroxocobalamin (blue trace), pET overexpressing the mutase and grown without hydroxocobalamin (green trace), or pET overexpressing the mutase and grown with hydroxocobalamin (red trace) are shown.

Figure 5 shows the three routes or biosynthetic pathways for the synthesis of methylmalonyl-CoA that can be engineered into yeast.

Figure 6 shows acyl-CoA analysis of *E. coli* overexpressing methylmalonyl-CoA mutase. The level of ^{3}H detected in fractions collected from HPLC of cell-free extracts from [^{3}H] ß-alanine-fed *E. coli* harboring either the pET control vector (solid trace) or pET overexpressing the mutase (dashed trace) is shown.

Figure 7 shows acyl-CoA analysis in *S. cerevisiae*. The level of ^{3}H detected in fractions collected from HPLC of cell-free extracts from [^{3}H] ß-alanine-fed *S. cerevisiae* after growth for 24 hours (solid trace), 48 hours (dashed trace) and 66 hours (dotted trace) is shown.

Figure 8 shows Common Cloning Cassette.

Figure 9 shows a general method for cloning genes into yeast expression vectors.--

Please replace the paragraph, beginning on page 9, line 1, with the following rewritten paragraph:

Each of the three polypeptide subunits of DEBS (DEBSI, DEBSII, and DEBSIII) contains 2 extender modules, DEBSI additionally contains the loading module. Collectively,

these proteins catalyze the condensation and appropriate reduction of 1 propionyl CoA starter unit and 6 methylmalonyl CoA extender units. Modules 1, 2, 5, and 6 contain KR domains; module 4 contains a complete set, KR/DH/ER, of reductive and dehydratase domains; and module 3 contains no functional reductive domain. Following the condensation and appropriate dehydration and reduction reactions, the enzyme bound intermediate is lactonized by the TE at the end of extender module 6 to form 6-dEB.

Please replace the paragraph, beginning on page 19, line 28, with the following rewritten paragraph:

A suitable propionyl CoA carboxylase (6.4.1.3) gene for purposes of the present invention can be isolated from *Streptomyces coelicolor* as reported in GenBank locus AF113605 (pccB), AF113604 (accA2) and AF113603 (accA1) by H. C. Gramajo and colleagues. The propionyl CoA carboxylase gene product requires biotin for activity. If the host cell does not make biotin, then the genes for biotin tranport can be transferred to the host cell. Even if the host cell makes or transports biotin, the endogenous biotin transferase enzyme may not have sufficient activity (whether due to specificity constraints or other reasons) to biotinylate the propionyl CoA carboxylase at the rate required for high level precursor synthesis. In this event, one can simply provide the host cell with a sufficiently active biotin transferase enzyme gene, or if there is an endogenous transferase gene, such as the *birA* gene in *E. coli*, one can simply overexpress that gene by recombinant methods. Many additional genes coding for propionyl CoA carboxylases, or acetyl CoA carboxylases with relaxed substrate specificity that includes propionate, have been reported and can be used as sources for this gene, as shown in the following table.

Please replace the paragraph, beginning on page 34, line 20, with the following rewritten paragraph:

Methods for generating libraries of polyketides have been greatly improved by cloning PKS genes as a set of three or more mutually selectable plasmids, each carrying a different wild-type or mutant PKS gene, then introducing all possible combinations of the plasmids with wild-type, mutant, and hybrid PKS coding sequences into the same host (see U.S. patent application Serial No. 60/129,731, filed 16 Apr. 1999, and PCT Pub. No. 98/27203, each of which is

incorporated herein by reference). This method can also incorporate the use of a KS1° mutant, which by mutational biosynthesis can produce polyketides made from diketide starter units (see Jacobsen *et al.*, 1997, *Science* **277**, 367-369, incorporated herein by reference), as well as the use of a truncated gene that leads to 12-membered macrolides or an elongated gene that leads to 16-membered ketolides. Moreover, by utilizing in addition one or more vectors that encode glycosyl biosynthesis and transfer genes, such as those of the present invention for megosamine, desosamine, oleandrose, cladinose, and/or mycarose (in any combination), a large collection of glycosylated polyketides can be prepared.

Please replace the paragraph, beginning on page 46, line 18, with the following rewritten paragraph:

As noted above, the hybrid PKS genes of the invention can be expressed in a host cell that contains the desosamine, megosamine, and/or mycarose biosynthetic genes and corresponding transferase genes as well as the required hydroxylase gene(s), which may be either *picK*, *megK*, or *eryK* (for the C-12 position) and/or *megF* or *eryF* (for the C-6 position). The resulting compounds have antibiotic activity but can be further modified, as described in the patent publications referenced above, to yield a desired compound with improved or otherwise desired properties. Alternatively, the aglycone compounds can be produced in the recombinant host cell, and the desired glycosylation and hydroxylation steps carried out *in vitro* or *in vivo*, in the latter case by supplying the converting cell with the aglycone, as described above.

Please delete figures and graphs on pages 58, 59, 62, 74, 82, 83, 84, and 87.

Please replace the paragraph, beginning at page 58, line 1, with the following rewritten paragraph:

--Figure 2 shows the construction of pSK – MUT, in which four PCR fragments were sequenced and pieced together to form the complete mutase gene in pSK-bluescript.--

Please replace the paragraph, beginning at page 59, line 3, with the following rewritten paragraph:

--In follow-up experiments, the specific activity of the mutase was determined and an in-depth CoA analysis was completed (as shown in Figure 3). The CoA levels in the cells were again analyzed using a *pan*D strain, which is a β-alanine auxotroph. ^{3}H-β-alanine was fed to the cells and incorporated into the acyl-CoAs, which were separated via HPLC and counted. The CoA pools for cell extracts with and without the mutase, as well as with and without hydroxocobalamin, were examined.--

Please replace the paragraph, beginning at page 62, line 2, with the following rewritten paragraph:

--Figure 4 shows the results of CoA analysis of *E. coli* overexpressing methylmalonyl-CoA mutase. The levels of ^{3}H detected in fractions collected from HPLC of cell-free extracts from ^{3}H ß-alanine-fed *E. coli* harboring either the pET control vector grown without hydroxocobalamin (black trace), pET grown with hydroxocobalamin (blue trace), pET overexpressing the mutase and grown without hydroxocobalamin (green trace), or pET overexpressing the mutase and grown with hydroxocobalamin (red trace) are shown.--

Please replace the paragraph, beginning at page 66, line 28, with the following rewritten paragraph:

--The sequence of the putative epimerase gene contained in cosmid 117-167-A7 was aligned to the N-terminal epimerase sequence already known. The several hundred base pairs downstream of this sequence were translated in all three frames and a stop codon in one of the frames was found that yielded a protein of the expected size. The entire sequence was used to search the protein database via BLAST analysis, and the sequence showed high homology to the sequence of a putative epimerase from *S. coelicolor* identified in accordance with the methods of the invention. PCR primers were designed based on the DNA sequence of the cloned *P. shermanii* epimerase and the gene was amplified from *P. shermanii* genomic DNA with *Nde*I and *Bam*HI sites at the 5'-end, an internal *Nde*I site was destroyed near the 5' end, and *Nhe*I and *Avr*II sites were introduced at the 3'-end. Following PCR, the 447 bp product was cloned into a Bluescript vector (143-6-11) and sequenced. Also, four additional sequencing primers were designed to provide several-fold coverage of the epimerase gene. The full epimerase gene

sequence provided in isolated and recombinant form by the present invention is shown below (SEQ ID NOS: 1 and 2).--

Please replace the paragraph, beginning at page 74, line 8, with the following rewritten paragraph:

--There are three routes or biosynthetic pathways for the synthesis of methylmalonyl-CoA that can be engineered into yeast, as shown in Figure 5. These pathways have been shown to produce methylmalonyl-CoA in *E. coli* and can be used to produce methylmalonyl-CoA in yeast. This example describes the identification of a system for methylmalonyl-CoA production in yeast, and a method for introducing it into the yeast chromosome.--

Please replace the paragraph, beginning at page 82, line 6, with the following rewritten paragraph:

--Figure 6 shows acyl-CoA analysis of *E. coli* overexpressing methylmalonyl-CoA mutase. The level of ^{3}H detected in fractions collected from HPLC of cell-free extracts from [^{3}H] ß-alanine-fed *E. coli* harboring either the pET control vector (solid trace) or pET overexpressing the mutase (dashed trace) is shown.--

Please replace the paragraph, beginning at page 83, line 12, with the following rewritten paragraph:

--Figure 7 shows acyl-CoA analysis in *S. cerevisiae*. The level of ^{3}H detected in fractions collected from HPLC of cell-free extracts from [^{3}H] ß-alanine-fed *S. cerevisiae* after growth for 24 hours (solid trace), 48 hours (dashed trace) and 66 hours (dotted trace) is shown. The yeast strain InvSc1 [3], grown in synthetic YNB media lacking pantothenate and ß-alanine, was used for acyl-CoA analysis. Yeast cultures starved of ß-alanine were fed [^{3}H] ß-alanine and the cultures were grown for 24, 48 and 66 hours at 30°C. Cells were disrupted with glass beads in the presence of 10% cold TCA and acyl-CoAs were separated by HPLC and quantified by scintillation counting. The yeast CoA pools were labeled with [^{3}H], but the extent of isotope dilution remains unclear. One can measure the specific activity of total CoA in these strains to ascertain the extent of isotope dilution.--

Please replace the paragraph, beginning at page 84, line 5, with the following rewritten paragraph:

--For PKS genes and initial studies of metabolic pathway genes, one can employ the analogous sets of bluescript cloning vectors and yeast 2 micron replicating shuttle vectors used in 6-MSA production [3]. With these vectors, yeast expression is driven by the alcohol dehydrogenase 2 (ADH2) promoter, which is tightly repressed by glucose and is highly active following glucose depletion that occurs after the culture reaches high density. Both vector sets have a "common cloning cassette" that contains, from 5' to 3', a polylinker (L1), the ADH2 (or other) promoter, a *Nde* I restriction site, a polylinker (L2), an ADH2 (or other) terminator, and a polylinker (L3). Due to excess restriction sites in the yeast shuttle vectors, genes of interest are first introduced into intermediate bluescript cloning vectors via the *Nde* I site, to generate the ATG start codon, and a downstream restriction site in the L2 polylinker that is common to the bluescript and yeast shuttle vectors (Figure 8). The promoter-gene cassette is then excised as an L1-L2 fragment and transferred to the yeast expression vector containing the transcriptional terminator.--

Please replace the paragraph, beginning at page 87, line 1, with the following rewritten paragraph:

--Figure 9 shows a general method for cloning genes into yeast expression vectors.--

In the Claims:

Please replace the presently pending claims with the following claims:

1. (Amended) A recombinant *E. coli* host cell comprising one or more expression vectors that comprise

a methylmalonyl CoA mutase gene selected from the group consisting of a *Propionibacterium shermanii* methylmalonyl CoA mutase gene and a *Streptomyces cinnamonensis* methylmalonyl CoA mutase gene, and

a *Propionibacterium shermanii* epimerase gene,